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(54) **Title:** PEPTIDE NUCLEIC ACID CONJUGATES

(57) **Abstract:** The present invention relates to peptide nucleic acid (PNA) conjugates, to methods for their preparation, to compositions comprising the conjugates and to the use of these conjugates as medicaments and their use in therapy e.g. in the treatment of infections.The invention further concerns cell penetrating peptides and methods of conjugating the peptides with PNA.

# PEPTIDE NUCLEIC ACID CONJUGATES

The present invention relates to peptide nucleic acid (PNA) conjugates, to methods for their preparation, to compositions comprising the conjugates and to the use of these conjugates as medicaments and their use in therapy e.g. in the treatment of infections.

The invention further concerns cell penetrating peptides and methods of conjugating the peptides with PNA.

## BACKGROUND OF THE INVENTION

Antisense agents offer a novel strategy in combating diseases, as well as opportunities to employ new chemical classes in the drug design.

Oligonucleotides can interact with native DNA and RNA in several ways. One of these is duplex formation between an oligonucleotide and a single stranded nucleic acid. Another is triplex formation between an oligonucleotide and double stranded DNA to form a triplex structure.

Antisense oligonucleotide drug formulations against viral and disease causing human genes are progressing through clinical trials. Efficient antisense inhibition of bacterial genes also could have wide applications; however, there have been few attempts to extend antisense technology to bacteria.

Peptide nucleic acids (PNA) are compounds that in certain respects are similar to oligonucleotides and their analogs and thus may mimic DNA and RNA. In PNA, the deoxyribose backbone of oligonucleotides has been replaced by a pseudo-peptide backbone (Nielsen et al. 1991 (1)), (Fig. 1). Each subunit, or monomer, has a naturally occurring or non-naturally occurring nucleobase attached to this backbone. One such backbone is constructed of repeating units of N-(2-aminoethyl)glycine linked through amide bonds. PNA hybridises with complementary nucleic acids through Watson and Crick base pairing and helix formation (Egholm et al. 1993 (2)).

The Pseudo-peptide backbone provides superior hybridization properties (Egholm et al. 1993 (2)), resistance to enzymatic degradation (Demidov et al. 1994 (3)) and access to a variety of chemical modifications (Nielsen and Haaima 1997 (4)).

PNA binds both DNA and RNA to form PNA/DNA or PNA/RNA duplexes. The resulting PNA/DNA or PNA/RNA duplexes are bound with greater affinity than corresponding DNA/DNA or DNA/RNA duplexes as determined by Tm's. This high thermal stability might be attributed to the lack of charge repulsion due to the neutral backbone in PNA. In addition to increased affinity, PNA has also been shown to bind to DNA with increased specificity. When a PNA/DNA duplex mismatch is melted relative to the DNA/DNA duplex, there is seen an 8 to 20°C drop in the Tm.

The stability expressed as the melting temperature ($T_m$), defined as the temperature at which 50% of the complexes have been dissociated, is determined as described by Arghya Ray et al (14).

Furthermore, homopyrimidine PNA oligomers form extremely stable $PNA_2$-DNA triplexes with sequence complementary targets in DNA or RNA oligomers. Finally, PNA's may bind to double stranded DNA or RNA by helix invasion.

An advantage of PNA compared to oligonucleotides is that the PNA polyamide backbone (having appropriate nucleobases or other side chain groups attached thereto) is not recognised by either nucleases or proteases and are thus not cleaved. As a result, PNA's are resistant to degradation by enzymes unlike nucleic acids and peptides.

For antisense application, target bound PNA can cause steric hindrance of DNA and RNA polymerases, reverse transcription, telomerase and of the ribosome's (Hanvey et al. 1992 (5), Knudsen et a. 1996 (6), Good and Nielsen 1998 (11,12)).

A general difficulty when using antisense agents is cell uptake. A variety of strategies to improve uptake can be envisioned and there are reports of improved uptake into eukaryotic cells using lipids (Lewis et al. 1996 (7)), encapsulation (Meyer

et al. 1998 (8)) and carrier strategies (Nyce and Metzger 1997 (9), Pooga et al, 1998 (10)).

WO 99/05302 discloses a PNA conjugate consisting of PNA and the transporter peptide transportan, which peptide may be used for transport cross a lipid membrane and for delivery of the PNA into interactive contact with intracellular polynucleotides.

US-A-5 777 078 discloses a pore-forming compound which comprises a delivery agent recognising the target cell and being linked to a pore-forming agent, such as a bacterial exotoxin. The compound is administered together with a drug such as PNA.

As an antisense agent for microorganisms, PNA may have unique advantages. It has been demonstrated that PNA based antisense agents for bacterial application can control cell growth and growth phenotypes when targeted to Escherichia coli rRNA and mRNA (Good and Nielsen 1998a,b (39,40) and WO 99/13893).

However, none of these disclosures discuss ways of transporting the PNA across the bacterial cell wall and membrane.

Furthermore, for bacterial application, poor uptake is expected, because bacteria have stringent barriers against foreign molecules and antisense oligomer containing nucleobases appear to be too large for efficient uptake. The results obtained by Good and Nielsen (1998a,b (39,40)) indicate that PNA oligomers enter bacterial cells poorly by passive diffusion across the lipid bilayers.

US-A-5 834 430 discloses the use of potentiating agents, such as short cationic peptides in the potentiation of antibiotics. The agent and the antibiotic are co-administered.

WO 96/11205 discloses PNA conjugates, wherein a conjugated moiety may be placed on terminal or non terminal parts of the backbone of PNA in order to

functionalise the PNA. The conjugated moieties may be reporter enzymes or molecules, steroids, carbohydrate, terpenes, peptides, proteins, etc. It is suggested that the conjugates among other properties may possess improved transfer properties for crossing cellular membranes. However, WO 96/11205 does not disclose conjugates, which may cross bacterial membranes.

WO 98/52614 discloses a method of enhancing transport over biological membranes, e.g. a bacterial cell wall. According to this publication, biological active agents such as PNA may be conjugated to a transporter polymer in order to enhance the transmembrane transport. The transporter polymer consists of 6-25 subunits; at least 50% of which contain a guanidino or amidino sidechain moiety and wherein at least 6 contiguous subunits contain guanidino and/or amidino sidechains. A preferred transporter polymer is a polypeptide containing 9 arginine.

WO 01/27261 discloses conjugates of cationic peptides and PNA.

## SUMMARY OF THE INVENTION

The present invention concerns a new strategy for combating bacteria. It has previously been shown that antisense PNA can inhibit growth of bacteria. However, due to a slow diffusion of the PNA over the bacterial cell wall a practical application of the PNA as an antibiotic has not been possible previously. According to the present invention, a practical application in tolerable concentration may be achieved by modifying the PNA by linking a peptide or peptide-like sequence, which enhances the activity of the PNA.

Surprisingly, it has been found out that by incorporating a peptide, an enhanced anti-infective effect can be observed. The important feature of the modified PNA molecules seems to be a pattern comprising in particular positively charged and lipophilic amino acids or amino acid analogues. An anti-infective effect is found with different orientation of the peptide in relation to the PNA-sequence.

Thus, the present invention concerns a modified PNA molecule of formula (I):

TP-L-PNA (I)

wherein TP is a transporter peptide, L is a bond or a linker and PNA is a peptide nucleic acid (PNA) oligomer of from 4 to 35 monomers.

PNA oligomers consisting of from 4 to 35 monomers of the present invention targeted to specific sequences of the messenger RNA of specific genes can be used as antisense reagents and drugs for down regulation of the expression of these genes in molecular biology and medicine. The PNA oligomers may be conjugated to carrier peptides to facilitate cellular uptake. Medical applications include treatment of bacterial and viral infections, cancer, metabolic diseases, immunological disorders etc.

PNA oligomers may also be used as hybridization probes in genetic diagnostics as exemplified by in situ hybridization, real time PCR monitoring and PCR modulation by "PNA-clamping".

Finally, PNA oligomers that bind to targets in double stranded DNA by a variety of mechanisms (e.g. triplex binding, duplex invasion, triplex invasion and double duplex invasion) may be developed into antigene drugs by targeting specific sequences of specific genes. In this way the expression of the targeted gene can be inhibited (or in desired cases activated), and the level of a disease related gene product thereby regulated.

In another preferred embodiment of the invention the modified PNA molecules of formula I are used in the treatment or prevention of infections caused by methicillin-resistant and methicillin-vancomycin-resistant *Staphylococcus aureus* or in the treatment or prevention of infections caused by vancomycin-resistant enterococci such as *Enterococcus faecalis* and *Enterococcus faecium.*

The present invention further concerns cell penetrating peptides and methods of conjugating the peptide with PNA.

In one embodiment, the peptide of the present invention contains from 2 to 60 modified amino acids.

The modified amino acids can be negatively, non-charged or positively charged and based upon naturally occurring or unnatural, i.e. rearranged or modified amino acids.

In a preferred embodiment of the invention the peptide contains from 2 to 18 modified amino acids, most preferred from 5 to 15 modified amino acids.

Preferred peptides are transporter peptides selected from table 1 and 2.

Further preferred peptides are transporter peptides selected from table 3a and 3b as well as transporter peptides of the formula $X_1X_2X_2X_2X_2X_1X_1X_2X_2X_1$, $X_1X_2X_2X_1X_1X_2X_2X_2X_2X_1$, or $X_1X_2X_2X_1X_2X_1X_1X_2X_2X_2$, wherein $X_1$ is K, R, E, D or H and $X_2$ is F, Y, I, L, V or A.

The peptide is linked to the PNA sequence via the amino (N-terminal) or carboxy (C-terminal) end.

In a preferred embodiment the peptide is linked to the PNA sequence via the carboxy end.

Within the present invention, the compounds of formula I may be prepared in the form of pharmaceutically acceptable salts, especially acid-addition salts, including salts of organic acids and mineral acids. Examples of such salts include salts of organic acids such as formic acid, fumaric acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, succinic acid, malic acid, tartaric acid, citric acid, benzoic acid, salicylic acid and the like. Suitable inorganic acid-addition salts include salts of hydrochloric, hydrobromic, sulphuric and phosphoric acids and the like. Further examples of pharmaceutically acceptable inorganic or organic acid addition salts include the pharmaceutically acceptable salts listed in Journal of

Pharmaceutical Science, Berge et al, 66, 2 (1977) (13) which are known to the skilled artisan.

Also intended as pharmaceutically acceptable acid addition salts are the hydrates, which the present compounds are able to form.

The acid addition salts may be obtained as the direct products of compound synthesis. In the alternative, the free base may be dissolved in a suitable solvent containing the appropriate acid, and the salt isolated by evaporating the solvent or otherwise separating the salt and solvent.

The compounds of this invention may form solvates with standard low molecular weight solvents using methods known to the skilled artisan.

In another aspect of the invention the modified PNA molecules are used in the manufacture of medicaments for the treatment or prevention of infectious diseases or for disinfecting non-living objects.

In a further aspect, the invention concerns a composition for treating or preventing infectious diseases or disinfecting non-living objects.

In yet another aspect, the invention concerns the treatment or prevention of infectious diseases or treatment of non-living objects.

In yet a further aspect, the present invention concerns a method of identifying specific advantageous antisense PNA sequences, which may be used in the modified PNA molecule according to the invention.

## BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows the chemical structure of DNA and PNA oligomers.

FIGURE 2 shows the chemical structures of the different succinimidyl based linking groups used in the conjugation of the Peptide and PNA

DETAILED DESCRIPTION OF THE INVENTION

The PNA molecule is connected to the peptide moiety through a direct binding or through a linker. A variety of linking groups can be used to connect the PNA with the peptide.

Linking groups are described in WO 96/11205, WO98/52614 and WO 01/27261, the content of which are hereby incorporated by reference.

Some linking groups may be advantageous in connection with specific combinations of PNA and peptide.

Linking groups may be selected from compounds of table 2A, 2B or 2C.

Any of these compounds may be used as a single linking group or together with more groups in creating a suitable linker. Further, the different linking groups may be combined in any order and number in order to obtain different functionalities in the linker arm.

Preferred linking groups are ADO (8-amino-3,6-dioxaoctanoic acid), SMCC (succinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate) AHEX or AHA (6-aminohexanoic acid), 4-aminobutyric acid, 4-aminocyclohexylcarboxylic acid, LCSMCC (succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxy-(6-amido-caproate), MBS (succinimidyl m-maleimido-benzoylate), EMCS (succinimidyl N-ε-maleimido-caproylate), SMPH (succinimidyl 6-(β-maleimido-propionamido) hexanoate, AMAS (succinimidyl N-(α-maleimido acetate), SMPB (succinimidyl 4-(p-maleimidophenyl)butyrate), β.ALA (β-alanine), PHG (Phenylglycine), ACHC (4-aminocyclohexanoic acid), β.CYPR (β-(cyclopropyl) alanine) and ADC (amino dodecanoic acid).

Any of these groups may be used as a single linking group or together with more groups in creating a suitable linker. Further, the different linking groups may be combined in any order and number in order to obtain different functionalities in the linker arm.

In a preferred embodiment the linking group is a combination of the β.ALA linking group or the ADO linking group with any of the other above mentioned linking groups.

Thus, preferred linkers are -achc-β.ala-, -achc-ado-, -lcsmcc-β.ala-, -mbs-β.ala-, -emcs-β.ala-, -lcsmcc-ado-, -mbs-ado-, -emcs-ado- or –smph-ado-.

Further preferred linking groups are linkers selected from the group of pFPhe (4-fluoro Phenylalanine), pnPhe (4-nitro Phenylalanine), chg (cyclohexyl Glycine), aha (6-amino-hexanoic acid), Gly (Glycine), b.Ala (β-alanine), achc (Cis-4-aminocyclohexanoic acid), cha (β-cyclohexyl alanine), PheGly (Phenylglycine), g.abu (4-aminobutanoic acid), b.cypr (β-cyclopropyl alanine), m.achc (Cis-4-amino-cyclohexaneacetic acid), F5Phe (Pentafluoro-Phenylalanine), pmba (4-aminomethyl-benzoic acid), ado ([2-(N-2-amino ethoxy)ethoxy] acetic acid); Nle (Norleucine), Nva (Norvaline), smcc (succinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate), 4-aminobutyric acid, 4-aminocyclohexylcarboxylic acid, lcsmcc (succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxy-(6-amido-caproate), mbs (succinimidyl m-maleimido-benzoylate), emcs (succinimidyl N-ε-maleimido-caproylate), smph (succinimidyl 6-(β-maleimido-propionamido) hexanoate, amas (succinimidyl N-(α-maleimido acetate), smpb (succinimidyl 4-(p- maleimidophenyl)butyrate) and adc (amino dodecanoic acid).

Any of these groups may be used as a single linking group or together with more groups in creating a suitable linker. Further, the different linking groups may be combined in any order and number in order to obtain different functionalities in the linker arm.

In a preferred embodiment the linking group is a combination of two or three of the above mentioned linkers.

Preferred combinations of linkers are: pFPhe-cha, b.cypr-aha, m.machc-b.cypr, achc-b.Ala, pFPhe-pFPhe, 5Phe-pFPhe, b.cypr-g.abu, PheGly-g.abu, achc-g.abu or AcBB in combination with any of the other linkers selected from table 2A, 2B or 2C.

Further preferred linkers are -Gly-, -Gly Gly- or -Gly Gly Gly-.

The peptide is normally linked to the PNA sequence via the amino or carboxy end. However, the PNA sequence may also be linked to an internal part of the peptide or the PNA sequence is linked to a peptide via both the amino and the carboxy end.

By the terms "cationic amino acids and amino acid analogues" and "positively charged amino acids and amino acid analogues" are to be understood any natural or non-natural occurring amino acid or amino acid analogue which have a positive charge at physiological pH. Similarly the term "non-charged amino acids or amino acid analogs" is to be understood any natural or non-natural occurring amino acids or amino acid analogs which have no charge at physiological pH.

Among the positively charged amino acids and amino acid analogs may be mentioned lysine (Lys, K), arginine (Arg, R), diamino butyric acid (DAB) and ornithine (Orn). The skilled person will be aware of further positively charged amino acids and amino acid analogs.

Among the non-charged amino acids and amino acid analogs may be mentioned the natural occurring amino acids alanine (Ala, A), valine (Val, V), leucine (Leu, L), isoleucine (Ile, I), proline (Pro, P), phenylanaline (Phe, F), tryptophan (Trp, W), methionine (Met, M), glycine (Gly, G), serine (Ser, S), threonine (Thr, T), cysteine (Cys, C), tyrosine (Tyr, Y), asparagine (Asn, N) and glutamine (Gln, Q), the non-natural occurring amino acids 2-aminobutyric acid, β-cyclohexylalanine, 4-chlorophenylalanine, norleucine and phenylglycine. The skilled person will be aware of further non-charged amino acids and amino acid analogs.

Preferably, the non-charged amino acids and amino acid analogs are selected from the natural occurring non-polar amino acids Ala, Val, Leu, Ile, Phe, Trp and Met or the non-natural occurring non-polar amino acids β-cyclohexylalanine, 4-chlorophenylalanine and norleucine.

The term "functionally similar moiety" is defined as to cover all peptide-like molecules, which functionally mimic the Peptide as defined above and thus impart to the PNA molecule the same advantageous properties as the peptides comprising natural and non-natural amino acids as defined above.

The modified PNA molecule according to the present invention comprises a PNA oligomer of a sequence, which is complementary to at least one target nucleotide sequence in a microorganism, such as a bacterium. The target may be a nucleotide sequence of any RNA, which is essential for the growth, and/or reproduction of the bacteria. Alternatively, the target may be a gene encoding a factor responsible for resistance to antibiotics. In a preferred embodiment, the functioning of the target nucleotide sequence is essential for the survival of the bacteria and the functioning of the target nucleic acid is blocked by the PNA sequence, in an antisense manner.

The binding of a PNA strand to a DNA or RNA strand can occur in one of two orientations, anti-parallel or parallel. As used in the present invention, the term complementary as applied to PNA does not in itself specify the orientation parallel or anti-parallel. It is significant that the most stable orientation of PNA/DNA and PNA/RNA is anti-parallel. In a preferred embodiment, PNA targeted to single strand RNA is complementary in an anti-parallel orientation.

In a another preferred embodiment of the invention a bis-PNA consisting of two PNA oligomers covalently linked to each other is targeted to a homopurine sequence (consisting of only adenine and/or guanine nucleotides) in RNA (or DNA), with which it can form a $PNA_2$-RNA ($PNA_2$-DNA) triple helix.

In another preferred embodiment of the invention, the PNA contains from 5 to 20 nucleobases, in particular from 7-15 nucleobases, and most particular from 8 to 12 nucleobases.

Peptide Nucleic Acids are described in WO 92/20702 and WO 92/20703, the content of which is hereby incorporated by reference.

In a preferred embodiment of the PNA the backbone is aminoethylglycine as shown in Figure 1.

In another aspect of the present invention, the modified PNA molecules can be used to identify preferred targets for the PNA. Based upon the known or partly known genome of the target micro-organisms, e.g. from genome sequencing or cDNA libraries, different PNA sequences can be constructed and linked to a peptide and thereafter tested for its anti-infective activity. It may be advantageous to select PNA sequences shared by as many micro-organisms as possible or shared by a distinct subset of micro-organisms, such as for example Gram-negative or Gram-positive bacteria, or shared by selected distinct micro-organisms or specific for a single micro-organism.

ANTISENSE TARGETS

Potential target genes may be chosen based on the knowledge of bacterial physiology. A target gene may be found among those involved in one of the major process complexes: cell division, cell wall synthesis, protein synthesis (translation) and nucleic acid synthesis, fatty acid metabolism and gene regulation. A target gene may also be involved in antibiotic resistance.

A further consideration is that some physiological processes are primarily active in dividing cells whereas others are running under non-dividing circumstances as well.

Known target proteins in cell wall biosynthesis are penicillin binding proteins, PBPs, the targets of, e.g., the beta-lactam antibiotic penicillin. They are involved in the final stages of cross-linking of the murein sacculus.

*E. coli* has 12 PBPs, the high molecular weight PBPs: PBP1a, PBP1b, PBP1c, PBP2 and PBP3, and seven low molecular weight PBPs, PBP 4-7, DacD, AmpC and AmpH. Only the high molecular weight PBPs are known to be essential for growth and have therefore been chosen as targets for PNA antisense.

Methicillin sensitive *S. aureus* (MSSA) has four PBPs, PBP1-4, whereas methicillin resistant *S. aureus* (MRSA) has an additional PBP, PBP2' (PBP2a) encoded by the *mecA* gene. Recently, an additional PBP, PBP2b encoded by the pbpF gene, has been identified in *S. aureus* (Komatsuzawa et al., (15)).

Experiments have shown that PBP1 or PBP1 in combination with PBP2 or PBP3, is essential for cell viability. PBP4 is nonessential.

Characteristic for the *S. aureus* peptidoglycan is the pentaglycine side chain that connects L-Lys of the pentapeptide bound to N-acetylmuramic acid to the D-Ala in position 4 of the neighbouring pentapeptide. Apart from cross-linking and thus cell wall stability, the pentaglycine serves as attachment site for staphylococcal surface proteins, which play an important role in adhesion and pathogenicity. Inhibition of pentaglycine side chain formation also reduces methicillin resistance. Even though the synthesis of PBP2' is not affetced, this leads to β-lactam hypersusceptibility. PBP2' mediated methicillin resistance is dependend on the presence of the pentaglycine.

The pentaglycine is synthesised by the sequential activity of FmhB, FemA and FemB proteins. FmhB is supposed to be a lethal target, i.e. its activity is essential for bacterial growth. As the first enzyme FmhB plays a key role in the synhtesis of the pentaglycine and thus cell wall stability, pathogenicity and methicillin resistance.

FemA is essential for cell growth in the presence of β-lactam antibiotics and for expression of methicillin resistance. FemB is also involved in methicillin resistance. *femAB* null mutants are hardly viable, suggesting that at least in combination these proteins may be essential for methicillin sensiticve cells. The *femB* gene (ORF 419) lies next to the *femA* gene, both forming the *femAB* operon. FemB is also involved in methicillin resistance.

Protein biosynthesis is an important process throughout the bacterial cell cycle. Therefore, the effect of targeting areas in the field of protein biosynthesis is not dependent on cell division.

Both DNA and RNA synthesis are target fields for antibiotics. A known target protein in DNA synthesis is gyrase. Gyrase acts in replication, transcription, repair and restriction. The enzyme consists of two subunits, both of which are candidate targets for PNA.

Examples of potential targets primarily activated in dividing cells are *rpoD, gyrA, gyrB*, (transcription), *mrcA* (*ponA*), *mrcB* (*ponB, pbpF*), *mrdA, ftsI* (*pbpB*) (Cell wall biosynthesis), *ftsQ, ftsA* and *ftsZ* (cell division).

Examples of potential targets also activated in non-dividing cells are *infA, infB, infC, tufA/tufB, tsf, fusA, prfA, prfB, and prfC,* (Translation).

Other potential target genes are antibiotic resistance-genes. The skilled person would readily know from which genes to choose. Two examples are genes coding for beta-lactamases inactivating beta-lactam antibiotics, and genes encoding chloramphenicol acetyl transferase.

PNA's against such resistance genes could be used against resistant bacteria.

A further potential target gene is the acpP gene encoding the acyl carrier protein of *E. Coli*

ACP (acyl carrier protein) is a small and highly soluble protein, which plays a central role in type I fatty acid synthase systems. Intermediates of long chain fatty acids are covalently bound to ACP by a thioester bond between the carboxyl group of the fatty acid and the thiol group of the phosphopanthetheine prosthetic group.

ACP is one of the most abundant proteins in *E. coli*, constituting 0.25% of the total soluble protein (ca 6 x $10^4$ molecules per cell). The cellular concentration of ACP is regulated, and overproduction of ACP from an inducible plasmid is lethal to E. coli cells.

Examples of micro-organisms which may be treated in accordance with the present invention are Gram-positive organisms such as Streptococcus, Staphylococcus, Peptococcus, Bacillus, Listeria, Clostridium, Propionebacteria, Gram-negative bacteria such as Bacteroides, Fusobacterium, Escherichia, Klebsiella, Salmonella, Shigella, Proteus, Pseudomonas, Vibrio, Legionella, Haemophilus, Bordetella, Brucella, Campylobacter, Neisseria, Branhamella, and organisms which stain poorly or not at all with Gram's stain such as Mycobacteria, Treponema, Leptospira, Borrelia, Mycoplasma, Clamydia, Rickettsia and Coxiella,

Infectious diseases are caused by micro-organisms belonging to a very wide range of bacteria, viruses, protozoa, worms and arthropods and from a theoretical point of view PNA can be modified and used against all kinds of RNA in such micro-organisms, sensitive or resistant to antibiotics.

METHODS

The ability of the compounds of the present invention to inhibit bacterial growth may be measured in many ways, which should be clear to the skilled person. For the purpose of exemplifying the present invention, the bacterial growth is measured by the use of a microdilution broth method according to NCCLS guidelines. The present invention is not limited to this way of detecting inhibition of bacterial growth.

To illustrate one example of measuring growth and growth inhibition the following procedure may be used:

Bacterial strain: *E.coli* ATCC 25922

Media: 10% Mueller-Hinton broth, diluted with sterile water.
10% LB broth diluted with sterile water.
100% Mueller-Hinton broth.

Trays: 96 well trays, Costar # 3474, Biotech Line AS, Copenhagen. (Extra low sorbent trays are used in order to prevent / minimize adhesion of PNA to tray surface).

A logphase culture of *E.coli* is diluted with fresh preheated medium and adjusted to defined OD (here: Optical Density at 600 nm) in order to give a final concentration of $5x10^4$ and/or $5x10^5$ bacteria/ml medium in each well, containing 200 µl of bacterial culture. PNA is added to the bacterial culture in the wells in order to give final concentrations ranging from 300 nM to 1000 nM. Trays are incubated at 37°C by shaking in a robot analyzer, PowerWave$_x$, software KC$^{4,}$ Kebo.Lab, Copenhagen, for 16 h and optical densities are measured at 600 nM during the incubation time in order to record growth curves. Wells containing bacterial culture without PNA are used as controls to ensure correct inoculum size and bacterial growth during the incubation. Cultures are tested in order to detect contamination.

The individual peptide-L-PNA constructs have MW between approx. 4200 and 5000 depending on the composition. Therefore all tests were performed on a molar basis rather than on a weight/volume basis. However, assuming an average MW of the construct of 4500 a concentration of 500 nM equals 2.25 microgram/ml.

Growth inhibitory effect of PNA-constructs:

The bacterial growth in the wells is described by the lag phase i.e. the period until (before) growth starts, the log phase i.e. the period with maximal growth rate, the steady-state phase followed by the death phase. These parameters are used when evaluating the inhibitory (Minimal Inhibitory Concentration, abbr. MIC) and

bactericidal (Minimal Bactericidal Concentration, abbr. MBC) effect of the PNA on the bacterial growth, by comparing growth curves with and without PNA.

Total inhibition of bacterial growth is defined as: OD (16h) = OD (0h) or no visible growth according to NCCLS Guidelines

In an initial screening the modified PNA molecules are tested in the sensitive 10% medium assay. Positive results are then run in the 100% medium assay in order to verify the inhibitory effect in a more "real" environment (cf. the American guidelines (NCCLS)).

*In vivo* antibacterial efficacy is established by testing a compound of the invention in the mouse peritonitis/sepsis model as described by N. Frimodt-Møller et al. 1999, Chap. 14, Handbook of Animal Models of Infection.

For the *in vivo* efficacy experiment a number of female NMRI mice are inoculated with approximately $10^7$ cfu of *E. coli* ATCC 25922 intraperitoneally. Samples are drawn from blood and peritoneal fluid at 1, 2, 4 and 6 hrs post infection, and cfu/ml counted. 1 hr post infection the animals are treated once in groups with: 1. Gentamicin (38 mg/kg s.c.); 2. Ampicillin (550 mg/kg s.c.); 3. a compound of the invention (50 - 60 mg/kg i.v.); 4. no treatment.

Histamine release

It is commonly known that certain cationic peptides are able to induce segregation of histamine from mammalian cells. One example is the Mast Cell Degranulating peptide (MCD peptide), ref. Haberman, E.(18), a 22 residue peptide with seven cationic side chains.

Increased histamine levels following release from histamine rich cells is also known to induce adverse clinical symptoms mediated via the histamine receptors (H1, H2 and H3). For the same reason it is important to eliminate the histamine releasing activity.

Accordingly the compounds of the invention were tested according to the following method:

The test compound was added in isotonic solutions to aliquots of human blood in a ratio of 1:1. The mixed samples were incubated at 37 degrees centigrade for 5 min. Plasma was then separated by centrifugation and the plasma histamine concentration determined by high-performance liquid chromatography by the method of Ashmore, S.P. et al. as published in Journal of Chromatography, Biomedical Applications, 496 (1989) 435-440. Compound 48/80 was used as positive reference compound.

*In vitro* stability

Relevant tissues (liver, kidneys. lungs) were rapidly removed from sacrificed animals (NMRI mice or Sprague-Dawley rats) and immediately placed in 0.25 M sucrose at 0 °C for rapid cooling and removal of external blood. After cooling the tissue was dryed by blotting with paper, weighed and transfered to clean test tubes. To each tissue 0.25 M sucrose in water was added to a final concentration of 150 mg tissue/ml. The tissue was homogenised and centrifuged in a refrigerated centrifuge (4 °C) for 30 minut at 3000 rpm (corresponding to approx. 1000 x g). The supernatant was carefully decanted and transferred to plastic containers and stored at -80 °C pending juse. Volumes of 0.025 ml 0.1 M Tris buffer pH 7.4, 0.145 ml of water were mixed with 0.025 ml aliquots of the tissue homogenates. The mixtures were pre-incubated at 37 °C for 2 minutes, and then added 0.005 ml (~ 1000 ng) of the PNA test compound. After incubation for 15 minutes the enzymatic reactions was stopped by adding 0.300 ml of 16.6% ACN in 0.1% TFA in water. The test mixture was then transferred to an ice-water bath (0 °C), and subsequently centrifuged at 3000 rpm for 10 min (approx. 1000 x g) at 4 °C. Volumes of 0.200 ml supernatant was transferred to autosampler vials. Aliquots of 0.010 ml were injected into the HPLC system. Chromatographic separation was obtained on a Symmetry 300™ C18, 2.1 x 150 mm column (Waters) equipped with a Zorbax Eclipse XDB-C18 guard column (Agilent), using a linear gradient elusion of solvent A (0.1% TFA in water) and solvent B (0.1% TFA in acetonitrile) from 2% to 75% solvent B over 8

minutes. The column was operated at 50 °C; samples were in the autosampler at 5 °C. Solvent flow was 0.4 ml/min. Recoveries were based on peak areas, and calculated per mg protein, where the protein concentration in the homogenates was determined by the colorimetric method of BCA Protein Assay Reagens. Additional blind samples were incubated and analyzed as described for the test samples.

*In vivo* pharmacokinetics

The pharmacokinetics of the compounds was investigated in NMRI mice. The mice were dosed intravenously, perorally or subcutaneously with the test compound. Dose preparations were prepared in 5% glucose solution or other isotonic vehicles. Plasma samples were collected at intervals from 0 to 4 (24) hours after dosing. Intact test compound was extracted from plasma by a solid-phase extraction procedure and the plasma concentrations determined by HPLC analysis. The plasma half-life was calculated from the terminal part of the plasma concentration versus time curve. Areas under the plasma concentration versus time curve (AUC) were calculated by the trapezoidal method and the oral (or subcutaneous) bioavailability calculated as the ratio (AUC[p.o. (or s.c.) adm]/AUC[i.v. adm.])x100 %, adjusted for actual doses.

PHARMACEUTICAL COMPOSITIONS

In a further aspect of the present invention, the invention provides a composition for use in inhibiting growth or reproduction of infectious micro-organisms comprising a modified PNA molecule according to the present invention. In one embodiment, the inhibition of the growth of micro-organisms is obtained through treatment with either the modified PNA molecule alone or in combination with antibiotics or other anti-infective agents. In another embodiment, the composition comprises two or more different modified PNA molecules. A second modified PNA molecule can be used to target the same bacteria as the first modified PNA molecule or in order to target different bacteria. In the latter form, specific combinations of target bacteria may be selected to the treatment. Alternatively, the target can be one or more genes, which confer resistance to one or more antibiotics to one or more bacteria. In such a

treatment, the composition or the treatment further comprises the use of said antibiotic(s).

In another aspect, the present invention includes within its scope pharmaceutical compositions comprising, as an active ingredient, at least one of the compounds of the general formula I, or a pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable carrier or diluent.

Pharmaceutical compositions containing a compound of the present invention may be prepared by conventional techniques, e.g. as described in Remington: The Science and Practice of Pharmacy, Gennaro, A. R. (editor)19$^{th}$ Ed., 1995. The compositions may appear in conventional forms, for example capsules, tablets, aerosols, solutions, suspensions or topical applications.

Typical compositions include a compound of formula I or a pharmaceutically acceptable acid addition salt thereof, associated with a pharmaceutically acceptable excipient which may be a carrier or a diluent or be diluted by a carrier, or enclosed within a carrier which can be in the form of a capsule, sachet, paper or other container. In making the compositions, conventional techniques for the preparation of pharmaceutical compositions may be used. For example, the active compound will usually be mixed with a carrier, or diluted by a carrier, or enclosed within a carrier, which may be in the form of an ampoule, capsule, sachet, paper, or other container. When the carrier serves as a diluent, it may be solid, semi-solid, or liquid material, which acts as a vehicle, excipient, or medium for the active compound. The active compound can be adsorbed on a granular solid container for example in a sachet. Some examples of suitable carriers are water, salt solutions, alcohol's, polyethylene glycol's, polyhydroxyethoxylated castor oil, peanut oil, olive oil, glycine, gelatin, lactose, terra alba, sucrose, glucose, cyclodextrine, amylose, magnesium stearate, talc, gelatin, agar, pectin, acacia, stearic acid or lower alkyl ethers of cellulose, silicic acid, fatty acids, fatty acid amines, fatty acid monoglycerides and diglycerides, pentaerythritol fatty acid esters, polyoxyethylene, hydroxymethylcellulose and polyvinylpyrrolidone. Similarly, the carrier or diluent may include any sustained release material known in the art, such as glyceryl

monostearate or glyceryl distearate, alone or mixed with a wax. The formulations may also include wetting agents, emulsifying and suspending agents, preserving agents, sweetening agents, thickeners or flavoring agents. The formulations of the invention may be formulated so as to provide quick, sustained, or delayed release of the active ingredient after administration to the patient by employing procedures well known in the art.

The pharmaceutical compositions can be sterilized and mixed, if desired, with auxiliary agents, emulsifiers, salt for influencing osmotic pressure, buffers and/or coloring substances and the like, which do not deleteriously react with the active compounds.

The route of administration may be any route, which effectively transports the active compound to the appropriate or desired site of action, such as oral, nasal, rectal, pulmonary, transdermal or parenteral e.g. depot, subcutaneous, intravenous, intraurethral, intramuscular, intranasal, ophthalmic solution or an ointment, the parenteral or the oral route being preferred.

If a solid carrier is used for oral administration, the preparation may be tabletted placed in a hard gelatin capsule in powder or pellet form or it can be in the form of a troche or lozenge. If a liquid carrier is used, the preparation may be in the form of a suspension or solution in water or a non-aqueous media, a syrup, emulsion or soft gelatin capsules. Thickeners, flavorings, diluents, emulsifiers, dispersing aids or binders may be added.

For nasal administration, the preparation may contain a compound of formula I dissolved or suspended in a liquid carrier, in particular an aqueous carrier, for aerosol application. The carrier may contain additives such as solubilizing agents, e.g. propylene glycol, surfactants, absorption enhancers such as lecithin (phosphatidylcholine) or cyclodextrine, or preservatives such as parabenes.

For parenteral application, particularly suitable are injectable solutions or suspensions, preferably aqueous solutions with the active compound dissolved in polyhydroxylated castor oil.

Tablets, dragees, or capsules having talc and/or a carbohydrate carrier or binder or the like are particularly suitable for oral application. Preferable carriers for tablets, dragees, or capsules include lactose, cornstarch, and/or potato starch. A syrup or elixir can be used in cases where a sweetened vehicle can be employed.

In formulations for treatment or prevention of infectious diseases in mammals the amount of active modified PNA molecules used is determined in accordance with the specific active drug, organism to be treated and carrier of the organism.

Such mammals include also animals, both domestic animals, e.g. household pets, and non-domestic animals such as wildlife.

Usually, dosage forms suitable for oral, nasal, pulmonal or transdermal administration comprise from about 0.01 mg to about 500 mg, preferably from about 0.01 mg to about 100 mg of the compounds of formula I admixed with a pharmaceutically acceptable carrier or diluent.

In a still further aspect, the present invention relates to the use of one or more compounds of the general formula I, or pharmaceutically acceptable salts thereof for the preparation of a medicament for the treatment and/or prevention of infectious diseases.

In yet another aspect of the present invention, the present invention concerns a method of treating or preventing infectious diseases, which treatment comprises administering to a patient in need of treatment or for prophylactic purposes an effective amount of modified PNA according to the invention. Such a treatment may be in the form of administering a composition in accordance with the present invention. In particular, the treatment may be a combination of traditional antibiotic

treatment and treatment with one or more modified PNA molecules targeting genes responsible for resistance to antibiotics.

In yet a further aspect of the present invention, the present invention concerns the use of the modified PNA molecules in disinfecting objects other than living beings, such as surgery tools, hospital inventory, dental tools, slaughterhouse inventory and tool, dairy inventory and tools, barbers and beauticians tools and the like.

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EXAMPLES

All compounds are synthesized in a manifold system with 24 reactions vessel (6x4 PLS from Advanced Chemtech). All PNA monomers, HATU and linkers are commercial available.

The compounds prepared are characterized on MALDI and purified by HPLC.

The following examples are merely illustrative of the present invention and should not be considered limiting of the scope of the invention in any way.

The following abbreviations related to reagents are used in the experimental part.

TABLE 1. Monomers and reagents

(The monomers and the PNA sequences are stated in bold)

| | |
|---|---|
| **A** monomer | N-(2-Boc-aminoethyl)-N-($N^6$-(benzyloxycarbonyl)adenine-9-yl-acetyl)glycine |
| Boc | Tert butyloxycarbonyl |
| Boc-Lys(2-Cl-Z)-OH | N-α-Boc-N-ε-2-chlorobenzyloxycarbonyl-L-lysine |
| Boc-Phe-OH | Boc-phenylalanine |
| Boc-β-Ala-OH | Boc-β-Alanine |
| Boc-cha-OH | Boc-Cyclohexyl-Alanine |
| **C** monomer | N-(2-Boc-aminoethyl)-N-($N^4$-(benzyloxycarbonyl)cytosine-1-yl-acetyl)glycine |
| DCM | Dichloromethane |
| DIEA | N,N-diisopropylethylamine |
| DMF | N,N-dimethylformamide |
| DMSO | Dimethyl sulfoxide |
| **G** monomer | N-(2-Boc-aminoethyl)-N-($N^2$-(benzyloxycarbonyl)guanine-9-yl-acetyl)glycine |
| HATU | N-[(1-H-benzotriazole-1-yl)(dimethylamine)methylene]-N-methylmethanaminiumhexafluorophosphate N-oxide |
| HBTU | 2-(1-H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate |
| **J** monomer /nucleobase | N-(2-Boc-aminoethyl)-N-(N-2 -(benzyloxycarbonyl) isocytosine-5-yl-acetyl)glycine |
| MBHA resin | p-methylbenzhydrylamine resin |
| NMP | N-methyl pyrrolidone |
| **T** monomer | N-(2-Boc-aminoethyl)-N-(thymine-1-yl-acetyl)glycine |
| TFA | Trifluoroacetic acid |
| TFSMA | Trifluoromethanesulphonic acid |
| Tris | 2-amino-2-(hydroxymethyl)-1,3-propanediol |

The following abbreviations relating to linking groups are used in the experimental part:

Table 2A. Linking groups

(The linking groups as starting materials are indicated with capital letters whereas the linking groups in the finished peptide-PNA conjugate are indicated with small letters.)

| Abbreviation | Linker (IUPAC) |
|---|---|
| SMCC | Succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate |
| LCSMCC | Succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxy-(6-amido-caproate) |
| MBS | Succinimidyl m-maleimido-benzoylate |
| EMCS | Succinimidyl N-ε-maleimido-caproylate |
| SMPH | Succinimidyl 6-(β-maleimido-propionamido)hexanoate |
| AMAS | Succinimidyl N-(α-maleimido acetate) |
| SMPB | Succinimidyl 4-(p- maleimidophenyl)butyrate |
| β.ALA | β-alanine |
| PHG | Phenylglycine |
| ACHC | 4-aminocyclohexanoic acid |
| β.CYPR | β-(cyclopropyl) alanine |
| AHA, AHEX | 6-amino-hexanoic acid |
| ADO, AEEA-OH | ((2-aminoethoxy)ethoxy)acetic acid or 8-amino-3,6-dioxaoctanoic acid |
| ADC | Amino dodecanoic acid |

The linking groups containing a succinimidyl group are shown in Figure 2.

The following abbreviations that relates to the natural or non-natural occurring amino acids in the compounds of the invention are used (lower-case letters or underlining indicates the corresponding D-form of the amino acid):

The amino acids might also be used as linking groups

Table 2B. Linking groups

| | |
|---|---|
| Achc | *cis*-4-aminocyclohexane-carboxylic acid |
| Ado | (2-(N-2-aminoethoxy)ethoxy)-acetic acid |
| Aha | 6-Aminohexanoic acid |
| b.Ala | β-Alanine |
| b.Cypr | β-Cyclopropyl-L-alanine |
| β.F | 3-amino-3-phenyl propanoic acid |
| β.K | β-Lysine, 3,6-diaminohexanoic acid |
| Bip | Biphenyl-L-alanine |
| Bpa | 4-Benzoyl-L-phenylalanine |
| C | L-Cysteine |
| Cha | β-Cyclohexyl-L-alanine |
| D | L-Aspartic acid |
| E | L-Glutamic acid |
| F | L-Phenylalanine |
| F5Phe | Pentafluoro-L-phenylalanine |
| G | L-Glycine |
| g.Abu | 4-Aminobutyric acid |
| H | L-Histidine |
| I | L-Isoleucine |
| K | L-Lysine |
| L | L-Leucine |
| M | L-Methionine |
| m.Achc | 4-Amino-methylenecyclohexane-carboxylic acid |
| N | L-Asparagine |
| N(GlcNAc) | N-β-(2-acetamino-2-deoxy-β-glucopyranosyl)-L-aspargine |
| N.Me.Phe | N-methyl-L-phenylalanine |
| Nle | L-Norleucine |

| | |
|---|---|
| Nva | L-Norvaline |
| Orn | L-Ornithine |
| P | Proline |
| pFPhe | 4-Fluoro-L-phenylalanine |
| Phg | Phenyl-L-glycine |
| pMbA | 4-Aminomethylbenzoic acid |
| Q | L-Glutamine |
| R | L-Arginine |
| S | L-Serine |
| Sar | Sarcosine |
| T | L-Threonine |
| Tic | (S)-1,2,3,4-Tetrahydroisoquinoline-3-carboxylic acid |
| V | L-Valine |
| W | L-Tryptophan |
| Y | L-Tyrosine |

Table 2C. Linking groups

| | |
|---|---|
| Abu | 2-Aminobutyric acid |
| Abz | 4-aminobenzoic acid |
| AcBB | [acetyl-(2-amino-ethyl)-amino]-acetic acid |
| Adc | Amino dodecanic acid |
| Aeg | N-(2-aminoethyl)-N-(acetyl)glycine |
| Aib | α-Aminoisobutyric acid |
| Asu | α-Aminosuberic acid |
| ChG | Cyclohexyl-L-glycine |
| Cit | Citrulline |

| | |
|---|---|
| d.Pro | 3,4-Dehydroproline |
| Dab | L-2,4-Diaminobutyric acid |
| e.Ahx | 6-Aminohexanoic acid |
| Hci | Homocitrulline |
| Hlys | Homo-L-lysine |
| Hphe | Homo-L-phenylalanine |
| Hser | Homo-L-serine |
| Hyp | Hydroxyproline |
| Inp | Isonipecotic acid (4-piperidinecarboxylic acid) |
| Lys(C12) | N-ε-(dodecanic acid)-L-lysine |
| m.Achc | 4-Amino-methylenecyclohexane-carboxylic acid |
| N.Lys.G | N-(1-Aminobut-4-yl)-glycine |
| N.Me.Lys | N-Methyl-L-lycine |
| N.Phe.G | N-Benzyl-glycine |
| Nal | β-(2-Naphtyl)-L-alanine |
| Pen | Penicillamine |
| Phe(2-F) | 2-Fluoro-L-phenylalanine |
| Phe(3-F) | 3-Fluoro-L-phenylalanine |
| Phe(4-Me) | 4-Methyl-L-phenylalanine |
| Phe(pCl) | 4-Chloro-L-phenylalanine |
| pNPhe | 4-Nitro-L-phenylalanine |
| Pyr | Pyro-L-glutamine |
| Thi | β-(2-Thienyl)-L-alanine |
| Tyr(3,5-di-I) | 3,5-Diiodo-L-tyrosine |
| Val(bOH) | β-Hydroxy-L-valine |

The composition of mixtures of solvents is indicates on a volume basis, i.e. 30/2/10 (v/v/v).

Purification of peptide conjugates was done using a Gilson HPLC system and a preparative RP-C18 column from Vydac (22 x 250 mm, 10 µm). The column was equilibrated in a buffer containing 0.95% acetic acid and 5% ethanol and with a flow of 20 ml/min. Dissolved material was applied on the column and subsequently washed with 2 column volumes of 0.75 M ammonium acetate to remove residual TFA salt from the peptide. Bound material was then eluted using a linear gradient from 0.95% acetic acid and 5% (v/v) ethanol to 0.5% acetic acid and 50% (v/v) ethanol over 4 column volumes.

Purity of the collected fractions were analysed using LC-MS. The analytical separation was performed using a Waters Alliance system and with a Vydac column (2 x 150 mm, 5 µm). After pre-equilibrated of the column in 0.1% TFA and 5% acetonitrile, the sample was applied and separated using a linear gradient from 0.1% TFA and 5% acetonitrile to 0.1% TFA and 40% acetonitrile. Eluted components were detected using a diode array UV monitor followed by mass determination on a single quartropol instrument from Micromass. The MS was previously calibrated using the API Calibration (NaCsI) solution (700001593) from Waters. An intern peptide standard with a molecular weight of 4844 g/mol was included for each analysis series. Fractions with a purity of minimum 95% where collected and used for further evaluations.

EXAMPLE 1

Preparation of KFFKFFKFFK-b.ala-cha-**TTCAAACATAGT**-$NH_2$ (1)

Compound 1 is synthesized on 50 mg MBHA resin (loading 100 µmol/g) in a Teflon reactions vessel. Deprotection is done with 2x600 µL TFA/anisol 95/5 followed by washing with DCM, DMF, 5 % DIEA in DCM and DMF. The coupling mixture is 200

μL 0.26 M solution of monomer (Boc-PNA-T-monomer, Boc-PNA-C-monomer, Boc-PNA-A-monomer, Boc-PNA-G-monomer) in NMP mixed with 200 μL 0.5 M DIEA in pyridine and activated for 1 min. with 200 μL 0.202 M HATU in NMP. The coupling mixture for the linker and the peptide part is 200 μL 0.52 M NMP solution of amino acid (Boc-Lys(2-Cl-Z)-OH, Boc-Phe-OH, Boc-cha-OH and Boc-β-Ala-OH) mixed with 200 μL 1 M DIEA in NMP and activated for 1 min. with 200 μL 0.404 M HBTU in NMP. After the coupling the resin is washed with DMF, DCM and capped with 600 μL NMP/Pyridine/acetic anhydride 50/48/2. Washing with DCM, DMF and DCM terminates the synthesis cycle. The oligomer is deprotected and cleaved from the resin using "low-high" TFMSA. The resin is rotated for 1 h with 1 mL of TFA/dimethylsulfid/m-cresol/TFMSA 5.5/3/1/0.5. The solution is washed out and then washed with 600 μL of TFA and 1 mL of TFMSA/TFA/m-cresol 2/8/1 is added. The mixture is rotated for 1.5 h and then precipitated out in 8 mL diethylether.
The precipitate is washed with 8 mL of diethylether. The crude compound (1) is dissolved in water and purified by HPLC.

Purity after preparative HPLC 98%, Mw calculated: 4835 g/mol: found on MALDI: 4832 g/mol.

EXAMPLE 2 Identification of PNA transporter peptides

Transport of peptides into bacterial cells was determined by the method described by Vaara & Porro 1996 (16).

An antibiotic probe (Rifampicin, Sigma R-8883), which penetrate very poorly the intact enterobacterial outer membrane but which traverse the damaged membrane were used.

The antibiotic probe was used in decreasing concentrations together with the different peptides (10 μg/ml).

A transporter peptide was defined as a peptide resulting in a decrease in the Minimum Inhibitory Concentration (MIC) of the antibiotic. An MIC value for the

antibiotic probe was determined for all the test organisms, and the following organisms were used in the screening: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 10031) and *Enterococcus faecium* (ATCC 51559). The MIC determination was performed in Müeller-Hinton growth medium according to the NCCLS guidelines (NCCLS M7-A5 (17)). Antimicrobial activity of the peptides at the used concentration was determined as a control, and none of the shown peptides showed any antimicrobial activity when no antibiotic was present in the given experimental set-up.

Results:

The results are shown as (+) if the peptide results in a decrease in MIC for the Rifampicin and (–) if the MIC for Rifampicin was unchanged in the presence of peptide.

TABLE 1

| Peptide sequence | E. coli ATCC 25922 MIC µg/ml | Ps.aeru. ATCC 27853A MIC µg/ml | K.pneu-moniae ATCC 10031 MIC µg/ml | E.faecium ATCC 51559 MIC µg/ml |
|---|---|---|---|---|
| H-KFFKFFKFFK-NH2 | + | + | + | - |
| H-KFFKFFKFF-NH2 | + | - | + | - |
| H-TRSSRAGLQWPVGRVHRLLRK—OH | + | - | + | - |
| H-GIGKWLHSAKKFGKAFVGEIMNS-OH | + | + | + | - |
| H-GKPRPQQVPPRPPHPRL-OH | + | - | + | - |
| H-IKFLKFLKFL-OH | + | + | + | + |
| H-RQIKIWFQNRRMKWKK-OH | + | + | + | - |
| H-YRRRFSVSVR-OH | + | - | + | - |
| H-RRLSYSRRRF-OH | - | - | - | - |
| H-KKFKVKFVVKK-OH | + | + | + | - |
| H-INLKALAALAKKIL-OH | + | - | + | - |
| H-AGYLLGKINLKALAALAKKIL-OH | + | - | + | - |
| H-VFQFLGKIIHHVGNFVHGFSHVF-OH | - | - | + | - |
| H-LFKRHLKWKIIV-OH | + | + | + | - |

TABLE 2

| Peptide sequence | *E. coli* ATCC 25922<br><br>*MIC* *µg/ml* | *Ps.aerug.* ATCC 27853A<br><br>*MIC* *µg/ml* |
|---|---|---|
| H-CKKVVFKVKFKK-NH2 | + | + |
| HO-KFFKFFKFFK-H | + | + |
| H-CLRWWWPWRRK-OH | + | - |
| H-CFLPLIGRVLSGIL-OH | - | - |
| H-CYGRKKRRQRRR-OH | + | + |
| H-CRQIKIWFQNRRMKWKK-OH | + | + |
| H-KWKKKWKKGGC-OH | + | + |
| H-RWRRRWRRGGC-OH | + | + |
| H-crrrrrrrrr-OH[1)] | + | + |
| H-ckkwkmrrnqfwvkvqr-OH[1)] | | + |
| H-CGWTLNSAGYLLGKIN LKALAALAKKIL-OH | - | + |
| H-CALYLAIRRR-NH2 | - | + |
| H-CKFFKFFKFFK-NH2 | + | + |
| H-CRQIKIWFQNRRMKWKK-NH2 | + | + |
| H-VRRFPWWWPFLRR-NH2 | - | + |
| H-KFFKFFKFF-NH2 | + | + |
| H-KFFKFFKF-NH2 | + | + |
| H-KFFKFFK-NH2 | + | + |

[1)] D-amino acids

TABLE 3A - Further peptides identified as transporter peptides

KFLKLLKLFK
KLLKFFKFFK
KFAKAAKAFK
KFFKLLKFFK
KLFKLLKLFK
KFFKFFKLLK
KAFKAAKAFK
KVLKFFKFFK
KLLKFLKLFK
KFFKVLKFFK
KAAKFAKAFK
KFFKFFKVLK
KLLKLFKLFK
KLLKLLKFFK
KAAKAFKAFK
KLLKFFKLLK
KFFKLLKLLK
KVLKVLKFFK
KVLKFFKVLK
KFFKVLKVLK
KAAKAAKFFK
KAAKFFKAAK
KFFKAAKAAK
KAAKAAKAAK
KFFKFFKFFK
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KKFKFFFFFK
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KFKKFFFFKF
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KKVKLVLVLK
KKVKLVKLVL
VLKKVLKVLK
KVKLVLKVLK
KVKKLVLVLK
KVKKLVKLVL
VLVKKLKVLK
KVLVKLKVLK
KVLKKVLVLK
KVLKKVKLVL
VLVKLKKVLK
KVLVLKKVLK
KVLKVKLVLK
KVLKVKKLVL
VLVKLVKKLK
KVLVLVKKLK
KVLKVLVKLK
KVLKVLKKVL
VLVKLVKLKK
KVLVLVKLKK
KVLKVLVLKK
KVLKVLKVKL
KPFKFFKFFK
KFPKFFKFFK
KFFKPFKFFK
KFFKFPKFFK

KFFKFFKPFK
KFFKFFKFPK
KPFKPFKFFK
KPFKFPKFFK
KPFKFFKPFK
KPFKFFKFPK
KFFKPFKPFK
KFFKPFKFPK
KFPKFPKFPK
KFFKFFKFAK
KFFKFFKFPK
KKFKFFKFFG
KKFKFFKFFV
KFFKFFKFCK
KFFKFFKFSK
KKFKFFKFFH
KKFKFFKFFN
KFFKFFKFQK
KFFKFFKFTK
KKFKFFKFFI
KFFKFFKFGK
KFFKFFKFVK
KKFKFFKFFL
KFFKFFKFHK
KFFKFFKFNK
KKFKFFKFFM
KFFKFFKFIK
KKFKFFKFFA
KKFKFFKFFP
KFFKFFKFLK
KKFKFFKFFC
KKFKFFKFFS
KFFKFFKFMK

KKFKFFKFFQ
KKFKFFKFFT
KFFKFFKβFFK
KFFKFFKFβFK
KFFKFFKFFβK
KFFKFFKβFβFK
KFFKFFKβFFβK
KFFKFFKFβFβK
KFFKFFKβFβFβK

Wherein the underlining designates D-amino acids and βF and βK have the meanings as defined in the abbreviations table.

TABLE 3B - Further peptides identified as transporter peptides.

KFFFFKKFFK
KFFKKFFFFK
KFFKFKKFFF
KFFKLLKLLK
KFFKVLKLLK
KFFKLVKLLK
KFFKLLKVLK
KFFKLLKLVK
KFFKVVKLLK
KFFKVLKVLK
KFFKVLKLVK
KFFKLVKVLK
KFFKLVKLVK
KFFKLLKVVK
KFFKVVKVLK
KFFKVVKLVK
KFFKLVKVVK
KFFKVVKVVK

KFFKAVKVVK
KFFKVAKVVK
KFFKVVKAVK
KFFKVVKVAK
KFFKAAKVVK
KFFKAVKAVK
KFFKAVKVAK
KFFKVAKAVK
KFFKVAKVAK
KFFKVVKAAK
KLLKLLKFFK
KVLKLLKFFK
KLVKLLKFFK
KLLKVLKFFK
KLLKLVKFFK
KVVKLLKFFK
KVLKVLKFFK
KVLKLVKFFK
KLVKVLKFFK
KLVKLVKFFK
KLLKVVKFFK
KVVKVLKFFK
KVVKLVKFFK
KLVKVVKFFK
KVVKVVKFFK
KAVKVVKFFK
KVAKVVKFFK
KVVKAVKFFK
KVVKVAKFFK
KAAKVVKFFK
KAVKAVKFFK
KAVKVAKFFK
KVAKAVKFFK

KVAKVAKFFK
KVVKAAKFFK
KFFFFKKFFK
KLLLLKKFFK
KVLLLKKFFK
KLVLLKKFFK
KLLVLKKFFK
KLLLVKKFFK
KVVLLKKFFK
KVLVLKKFFK
KVLLVKKFFK
KLVVLKKFFK
KLVLVKKFFK
KLLVVKKFFK
KVVVLKKFFK
KVVLVKKFFK
KLVVVKKFFK
KVVVVKKFFK
KAVVVKKFFK
KVAVVKKFFK
KVVAVKKFFK
KVVVAKKFFK
KAAVVKKFFK
KAVAVKKFFK
KAVVAKKFFK
KVAAVKKFFK
KVAVAKKFFK
KVVAAKKFFK
KFFKKFFFFK
KFFKKLLLLK
KFFKKVLLLK
KFFKKLVLLK
KFFKKLLVLK

KFFKKLLLVK
KFFKKVVLLK
KFFKKVLVLK
KFFKKVLLVK
KFFKKLVVLK
KFFKKLVLVK
KFFKKLLVVK
KFFKKVVVLK
KFFKKVVLVK
KFFKKLVVVK
KFFKKVVVVK
KFFKKAVVVK
KFFKKVAVVK
KFFKKVVAVK
KFFKKVVVAK
KFFKKAAVVK
KFFKKAVAVK
KFFKKAVVAK
KFFKKVAAVK
KFFKKVAVAK
KFFKKVVAAK
KFFKFKKFFF
KFFKLKKLLL
KFFKVKKLLL
KFFKLKKVLL
KFFKLKKLVL
KFFKLKKLLV
KFFKVKKVLL
KFFKVKKLVL
KFFKVKKLLV
KFFKLKKVVL
KFFKLKKVLV
KFFKLKKLVV

KFFKVKKVVL
KFFKVKKLVV
KFFKLKKVVV
KFFKVKKVVV
KFFKAKKVVV
KFFKVKKAVV
KFFKVKKVAV
KFFKVKKVVA
KFFKAKKAVV
KFFKAKKVAV
KFFKAKKVVA
KFFKVKKAAV
KFFKVKKAVA
KFFKVKKVAA
KFFFFKFFK
KLLLLKFFK
KVLLLKFFK
KLVLLKFFK
KLLVLKFFK
KLLLVKFFK
KVVLLKFFK
KVLVLKFFK
KVLLVKFFK
KLVVLKFFK
KLVLVKFFK
KLLVVKFFK
KVVVLKFFK
KVVLVKFFK
KLVVVKFFK
KVVVVKFFK
KAVVVKFFK
KVAVVKFFK
KVVAVKFFK

KVVVAKFFK
KAAVVKFFK
KAVAVKFFK
KAVVAKFFK
KVAAVKFFK
KVAVAKFFK
KVVAAKFFK
KLLLKFFK
KVLLKFFK
KLVLKFFK
KLLVKFFK
KVVLKFFK
KVLVKFFK
KLVVKFFK
KVVVKFFK
KAVVKFFK
KVAVKFFK
KVVAKFFK
KAAVKFFK
KAVAKFFK
KVAAKFFK
KFFKFFFFK
KFFKLLLLK
KFFKVLLLK
KFFKLVLLK
KFFKLLVLK
KFFKLLLVK
KFFKVVLLK
KFFKVLVLK
KFFKVLLVK
KFFKLVVLK
KFFKLVLVK
KFFKLLVVK

KFFKVVVLK
KFFKVVLVK
KFFKLVVVK
KFFKVVVVK
KFFKAVVVK
KFFKVAVVK
KFFKVVAVK
KFFKVVVAK
KFFKAAVVK
KFFKAVAVK
KFFKAVVAK
KFFKVAAVK
KFFKVAVAK
KFFKVVAAK
KFFKLLLK
KFFKVLLK
KFFKLVLK
KFFKLLVK
KFFKVVLK
KFFKVLVK
KFFKLVVK
KFFKVVVK
KFFKAVVK
KFFKVAVK
KFFKVVAK
KFFKAAVK
KFFKAVAK
KFFKVAAK
KFFKFKFFF
KFFKLKLLL
KFFKVKLLL
KFFKLKVLL
KFFKLKLVL

KFFKLKLLV
KFFKVKVLL
KFFKVKLVL
KFFKVKLLV
KFFKLKVVL
KFFKLKVLV
KFFKLKLVV
KFFKVKVVL
KFFKVKLVV
KFFKLKVVV
KFFKVKVVV
KFFKAKVVV
KFFKVKAVV
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KFFKAKAVV
KFFKAKVAV
KFFKAKVVA
KFFKVKAAV
KFFKVKAVA
KFFKVKVAA
KFFKLKLL
KFFKVKLL
KFFKLKVL
KFFKLKLV
KFFKVKVL
KFFKVKLV
KFFKLKVV
KFFKVKVV
KFFKAKVV
KFFKVKAV
KFFKVKVA
KFFKAKAV

KFFKAKVA
KFFKVKAA

EXAMPLE 3

Preparation of PNA Conjugates

According to the method described in Example 1 PNA conjugates were prepared using the peptides from the tables 1, 2, 3a and 3b in Example 2.

The peptides were used in their full length as well as in the following truncated forms:

IKFLKFLKFL
IKFLKFLKF
IKFLKFLK
IKFLKFL
IKFLKF
IKFLK
KFLKFLKFL
FLKFLKFL
LKFLKFL
KFLKFL
FLKFL
KFLKFLKF
FLKFLKF
LKFLKF
KFLKF
FLKF
KFLKFLK
FLKFLK
LKFLK
KFLK

RQIKIWFQNRRMKWKK

FQNRRMKWKK

QNRRMKWKK

NRRMKWKK

RRMKWKK

INLKALAALAKKIL[2)]

[2)] Wherein 1-9 amino acid residues is deleted or variants thereof,

LFKRHLKWKIIV[3)]

[3)] Wherein 1-7 amino acid residues is deleted or variants thereof,

TRSSRAGLQWPVGRVHRLLRK[4)]

[4)]Wherein 1-17 amino acid residues is deleted or variants thereof,

RAGLQFPVG

RAGLQFAV

GKPRPQQVPPRPPHPRL[5)]

[5)] Wherein 1-13 amino acid residues is deleted or variants thereof,

PQQVPPRPPHPR

PQQKPPRPPHPR

PQQRPPRPPHPR

VPPRPPHPR

KPPRPPHPR

RPPRPPHPR

GIGKWLHSAKKFGKAFVGEIMNS[6)]

[6)] Wherein 1-18 amino acid residues is deleted or variants thereof,

GIGKWLHSAKKFG

GIGKWLHSAKKFGK

GIGKWLHSAKKFGKA

CRQIKIWFQNRRMKWKK[7)]

[7)] Wherein 1-13 amino acid residues is deleted or variants thereof,

VRRFPWWWPFLRR[8)]

[8)] Wherein 1-9 amino acid residues is deleted or variants thereof,

CLRWWWPWRRK[9)]

[9)] Wherein 1-7 amino acid residues is deleted or variants thereof,

CYGRKKRRQRRR[10)]

[10] Wherein 1-7 amino acid residues is deleted or variants thereof,

CRQIKIWFQNRRMKWKK[11]

[11] Wherein 1-12 amino acid residues is deleted or variants thereof,

crrrrrrrr[12]

[12] Wherein 1-6 amino acid residues is deleted or variants thereof,

KWKKKWKKGGC[13]

[13] Wherein 1-6 amino acid residues is deleted or variants thereof,

RWRRRWRRGGC[14]

[14] Wherein 1-6 amino acid residues is deleted or variants thereof,

CFLPLIGRVLSGIL[15]

[15] Wherein 1-9 amino acid residues is deleted or variants thereof,

ckkwkmrrnqfwvkvqr[16]

[16] Wherein 1-12 amino acid residues is deleted or variants thereof,

CGWTLNSAGYLLGKIN[17]

[17] Wherein 1-11 amino acid residues is deleted or variants thereof,

LKALAALAKKIL[18]

[18] Wherein 1-7 amino acid residues is deleted or variants thereof,

CALYLAIRRR[19]

[19] Wherein 1-5 amino acid residues is deleted or variants thereof,

YRRRFSVSVR[20]

[20] Wherein 1-5 amino acid residues is deleted or variants thereof,

RRLSYSRRRF[21]

[21] Wherein 1-5 amino acid residues is deleted or variants thereof,

KKFKVKFVVKK[22]

[22] Wherein 1-7 amino acid residues is deleted or variants thereof,

INLKALAALAKKIL[23]

[23] Wherein 1-9 amino acid residues is deleted or variants thereof,

AGYLLGKINLKALAALAKKIL[24]

[24] Wherein 1-16 amino acid residues is deleted or variants thereof,

VFQFLGKIIHHVGNFVHGFSHVF[25]

[25] Wherein 1-18 amino acid residues is deleted or variants thereof,

LFKRHLKWKIIV[26]

[26] Wherein 1-7 amino acid residues is deleted or variants thereof,

KFLKLLKLFK
KLLKFFKFFK
KFAKAAKAFK
KFFKLLKFFK
KLFKLLKLFK
KFFKFFKLLK
KAFKAAKAFK
KVLKFFKFFK
KLLKFLKLFK
KFFKVLKFFK
KAAKFAKAFK
KFFKFFKVLK
KLLKLFKLFK
KLLKLLKFFK
KAAKAFKAFK
KLLKFFKLLK
KFFKLLKLLK
KVLKVLKFFK
KVLKFFKVLK
KFFKVLKVLK
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KVLKVLVLKK
KVLKVLKVKL
KPFKFFKFFK
KFPKFFKFFK
KFFKPFKFFK
KFFKFPKFFK
KFFKFFKPFK
KFFKFFKFPK

KPFKPFKFFK
KPFKFPKFFK
KPFKFFKPFK
KPFKFFKFPK
KFFKPFKPFK
KFFKPFKFPK
KFPKFPKFPK
KFFKFFKFAK
KFFKFFKFPK
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KKFKFFKFFA
KKFKFFKFFP
KFFKFFKFLK
KKFKFFKFFC
KKFKFFKFFS
KFFKFFKFMK
KKFKFFKFFQ
KKFKFFKFFT

KFFKFFKβFFK
KFFKFFKFβFK
KFFKFFKFFβK
KFFKFFKβFβFK
KFFKFFKβFFβK
KFFKFFKFβFβK
KFFKFFKβFβFβK
KFFFFKKFFK
KFFKKFFFFK
KFFKFKKFFF
KFFKLLKLLK
KFFKVLKLLK
KFFKLVKLLK
KFFKLLKVLK
KFFKLLKLVK
KFFKVVKLLK
KFFKVLKVLK
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KAAKVVKFFK
KAVKAVKFFK
KAVKVAKFFK
KVAKAVKFFK
KVAKVAKFFK
KVVKAAKFFK
KFFFFKKFFK
KLLLLKKFFK
KVLLLKKFFK
KLVLLKKFFK
KLLVLKKFFK
KLLLVKKFFK

KVVLLKKFFK
KVLVLKKFFK
KVLLVKKFFK
KLVVLKKFFK
KLVLVKKFFK
KLLVVKKFFK
KVVVLKKFFK
KVVLVKKFFK
KLVVVKKFFK
KVVVVKKFFK
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KVVAVKKFFK
KVVVAKKFFK
KAAVVKKFFK
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KAVVAKKFFK
KVAAVKKFFK
KVAVAKKFFK
KVVAAKKFFK
KFFKKFFFFK
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KFFKKVLLLK
KFFKKLVLLK
KFFKKLLVLK
KFFKKLLLVK
KFFKKVVLLK
KFFKKVLVLK
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KFFKKLVLVK
KFFKKLLVVK
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KFFKKVVLVK
KFFKKLVVVK
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KFFKKAVAVK
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KFFKKVAAVK
KFFKKVAVAK
KFFKKVVAAK
KFFKFKKFFF
KFFKLKKLLL
KFFKVKKLLL
KFFKLKKVLL
KFFKLKKLVL
KFFKLKKLLV
KFFKVKKVLL
KFFKVKKLVL
KFFKVKKLLV
KFFKLKKVVL
KFFKLKKVLV
KFFKLKKLVV
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KFFKVKKLVV
KFFKLKKVVV
KFFKVKKVVV
KFFKAKKVVV
KFFKVKKAVV
KFFKVKKVAV
KFFKVKKVVA

KFFKAKKAVV
KFFKAKKVAV
KFFKAKKVVA
KFFKVKKAAV
KFFKVKKAVA
KFFKVKKVAA
KFFFFKFFK
KLLLLKFFK
KVLLLKFFK
KLVLLKFFK
KLLVLKFFK
KLLLVKFFK
KVVLLKFFK
KVLVLKFFK
KVLLVKFFK
KLVVLKFFK
KLVLVKFFK
KLLVVKFFK
KVVVLKFFK
KVVLVKFFK
KLVVVKFFK
KVVVVKFFK
KAVVVKFFK
KVAVVKFFK
KVVAVKFFK
KVVVAKFFK
KAAVVKFFK
KAVAVKFFK
KAVVAKFFK
KVAAVKFFK
KVAVAKFFK
KVVAAKFFK
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KVLLKFFK
KLVLKFFK
KLLVKFFK
KVVLKFFK
KVLVKFFK
KLVVKFFK
KVVVKFFK
KAVVKFFK
KVAVKFFK
KVVAKFFK
KAAVKFFK
KAVAKFFK
KVAAKFFK
KFFKFFFFK
KFFKLLLLK
KFFKVLLLK
KFFKLVLLK
KFFKLLVLK
KFFKLLLVK
KFFKVVLLK
KFFKVLVLK
KFFKVLLVK
KFFKLVVLK
KFFKLVLVK
KFFKLLVVK
KFFKVVVLK
KFFKVVLVK
KFFKLVVVK
KFFKVVVVK
KFFKAVVVK
KFFKVAVVK
KFFKVVAVK
KFFKVVVAK

KFFKAAVVK
KFFKAVAVK
KFFKAVVAK
KFFKVAAVK
KFFKVAVAK
KFFKVVAAK
KFFKLLLK
KFFKVLLK
KFFKLVLK
KFFKLLVK
KFFKVVLK
KFFKVLVK
KFFKLVVK
KFFKVVVK
KFFKAVVK
KFFKVAVK
KFFKVVAK
KFFKAAVK
KFFKAVAK
KFFKVAAK
KFFKFKFFF
KFFKLKLLL
KFFKVKLLL
KFFKLKVLL
KFFKLKLVL
KFFKLKLLV
KFFKVKVLL
KFFKVKLVL
KFFKVKLLV
KFFKLKVVL
KFFKLKVLV
KFFKLKLVV
KFFKVKVVL

KFFKVKLVV
KFFKLKVVV
KFFKVKVVV
KFFKAKVVV
KFFKVKAVV
KFFKVKVAV
KFFKVKVVA
KFFKAKAVV
KFFKAKVAV
KFFKAKVVA
KFFKVKAAV
KFFKVKAVA
KFFKVKVAA
KFFKLKLL
KFFKVKLL
KFFKLKVL
KFFKLKLV
KFFKVKVL
KFFKVKLV
KFFKLKVV
KFFKVKVV
KFFKAKVV
KFFKVKAV
KFFKVKVA
KFFKAKAV
KFFKAKVA
KFFKVKAA

CLAIMS

1. A modified PNA molecule of formula (I):

TP-L-PNA (I)

wherein TP is a transporter peptide, L is a bond or a linker and PNA is a peptide nucleic acid (PNA) oligomer of from 4 to 35 monomers.

2. A compound of claim 1 wherein the transporter peptide TP is of the formula $X_1X_2X_2X_2X_2X_1X_1X_2X_2X_1$, $X_1X_2X_2X_1X_1X_2X_2X_2X_2X_1$, or $X_1X_2X_2X_1X_2X_1X_1X_2X_2X_2$, wherein $X_1$ is K, R, E, D or H and $X_2$ is F, Y, I, L, V or A.

3. A compound of claim 1 wherein the transporter peptide TP is selected from:

IKFLKFLKFL
IKFLKFLKF
IKFLKFLK
IKFLKFL
IKFLKF
IKFLK
KFLKFLKFL
FLKFLKFL
LKFLKFL
KFLKFL
FLKFL
KFLKFLKF
FLKFLKF
LKFLKF
KFLKF
FLKF
KFLKFLK
FLKFLK

LKFLK

KFLK

RQIKIWFQNRRMKWKK

FQNRRMKWKK

QNRRMKWKK

NRRMKWKK

RRMKWKK

INLKALAALAKKIL[2)]

[2)] Wherein 1-9 amino acid residues is deleted or variants thereof,

LFKRHLKWKIIV[3)]

[3)] Wherein 1-7 amino acid residues is deleted or variants thereof,

TRSSRAGLQWPVGRVHRLLRK[4)]

[4)]Wherein 1-17 amino acid residues is deleted or variants thereof, especially the following truncations are preferred:

RAGLQFPVG

RAGLQFAV;

GKPRPQQVPPRPPHPRL[5)]

[5)] Wherein 1-13 amino acid residues is deleted or variants thereof, especially the following truncations are preferred:

PQQVPPRPPHPR

PQQKPPRPPHPR

PQQRPPRPPHPR

VPPRPPHPR

KPPRPPHPR

RPPRPPHPR;

GIGKWLHSAKKFGKAFVGEIMNS[6)]

[6)] Wherein 1-18 amino acid residues is deleted or variants thereof, especially the following truncations are preferred:

GIGKWLHSAKKFG

GIGKWLHSAKKFGK

GIGKWLHSAKKFGKA;

CRQIKIWFQNRRMKWKK[7)]

[7)] Wherein 1-13 amino acid residues is deleted or variants thereof,

VRRFPWWWPFLRR[8)]

[8)] Wherein 1-9 amino acid residues is deleted or variants thereof,

CLRWWWPWRRK[9)]

[9)] Wherein 1-7 amino acid residues is deleted or variants thereof,

CYGRKKRRQRRR[10)]

[10)] Wherein 1-7 amino acid residues is deleted or variants thereof,

CRQIKIWFQNRRMKWKK[11)]

[11)] Wherein 1-12 amino acid residues is deleted or variants thereof,

crrrrrrrr[12)]

[12)] Wherein 1-6 amino acid residues is deleted or variants thereof,

KWKKKWKKGGC[13)]

[13)] Wherein 1-6 amino acid residues is deleted or variants thereof,

RWRRRWRRGGC[14)]

[14)] Wherein 1-6 amino acid residues is deleted or variants thereof,

CFLPLIGRVLSGIL[15)]

[15)] Wherein 1-9 amino acid residues is deleted or variants thereof,

ckkwkmrrnqfwvkvqr[16)]

[16)] Wherein 1-12 amino acid residues is deleted or variants thereof,

CGWTLNSAGYLLGKIN[17)]

[17)] Wherein 1-11 amino acid residues is deleted or variants thereof,

LKALAALAKKIL[18)]

[18)] Wherein 1-7 amino acid residues is deleted or variants thereof,

CALYLAIRRR[19)]

[19)] Wherein 1-5 amino acid residues is deleted or variants thereof,

YRRRFSVSVR[20)]

[20)] Wherein 1-5 amino acid residues is deleted or variants thereof,

RRLSYSRRRF[21)]

[21)] Wherein 1-5 amino acid residues is deleted or variants thereof,

KKFKVKFVVKK[22)]

[22)] Wherein 1-7 amino acid residues is deleted or variants thereof,

INLKALAALAKKIL[23)]

[23)] Wherein 1-9 amino acid residues is deleted or variants thereof,

AGYLLGKINLKALAALAKKIL[24)]

[24)] Wherein 1-16 amino acid residues is deleted or variants thereof,

VFQFLGKIIHHVGNFVHGFSHVF[25)]

[25)] Wherein 1-18 amino acid residues is deleted or variants thereof,

LFKRHLKWKIIV[26)]

[26)] Wherein 1-7 amino acid residues is deleted or variants thereof.

4. A compound of claim 1 wherein the transporter peptide TP is selected from:

KFLKLLKLFK
KLLKFFKFFK
KFAKAAKAFK
KFFKLLKFFK
KLFKLLKLFK
KFFKFFKLLK
KAFKAAKAFK
KVLKFFKFFK
KLLKFLKLFK
KFFKVLKFFK
KAAKFAKAFK
KFFKFFKVLK
KLLKLFKLFK
KLLKLLKFFK
KAAKAFKAFK
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KVVAKFFK
KAAVKFFK
KAVAKFFK
KVAAKFFK
KFFKFFFFK
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KFFKLVLLK
KFFKLLVLK
KFFKLLLVK
KFFKVVLLK
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KFFKVLLVK
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KFFKVVVLK

KFFKVVLVK
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KFFKAVAVK
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KFFKAVVK
KFFKVAVK
KFFKVVAK
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KFFKAVAK
KFFKVAAK
KFFKFKFFF
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KFFKVKVL
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KFFKLKVV
KFFKVKVV
KFFKAKVV
KFFKVKAV
KFFKVKVA
KFFKAKAV
KFFKAKVA

KFFKVKAA

5. A transporter peptide selected from:

IKFLKFLKFL
IKFLKFLKF
IKFLKFLK
IKFLKFL
IKFLKF
IKFLK
KFLKFLKFL
FLKFLKFL
LKFLKFL
KFLKFL
FLKFL
KFLKFLKF
FLKFLKF
LKFLKF
KFLKF
FLKF
KFLKFLK
FLKFLK
LKFLK
KFLK
RQIKIWFQNRRMKWKK
FQNRRMKWKK
QNRRMKWKK
NRRMKWKK
RRMKWKK
INLKALAALAKKIL[2)]

[2)] Wherein 1-9 amino acid residues is deleted or variants thereof,

LFKRHLKWKIIV[3)]

[3)] Wherein 1-7 amino acid residues is deleted or variants thereof,

TRSSRAGLQWPVGRVHRLLRK[4)]

[4)]Wherein 1-17 amino acid residues is deleted or variants thereof,

RAGLQFPVG

RAGLQFAV

GKPRPQQVPPRPPHPRL[5)]

[5)] Wherein 1-13 amino acid residues is deleted or variants thereof,

PQQVPPRPPHPR

PQQKPPRPPHPR

PQQRPPRPPHPR

VPPRPPHPR

KPPRPPHPR

RPPRPPHPR

GIGKWLHSAKKFGKAFVGEIMNS[6)]

[6)] Wherein 1-18 amino acid residues is deleted or variants thereof,

GIGKWLHSAKKFG

GIGKWLHSAKKFGK

GIGKWLHSAKKFGKA

CRQIKIWFQNRRMKWKK[7)]

[7)] Wherein 1-13 amino acid residues is deleted or variants thereof,

VRRFPWWWPFLRR[8)]

[8)] Wherein 1-9 amino acid residues is deleted or variants thereof,

CLRWWWPWRRK[9)]

[9)] Wherein 1-7 amino acid residues is deleted or variants thereof,

CYGRKKRRQRRR[10)]

[10)] Wherein 1-7 amino acid residues is deleted or variants thereof,

CRQIKIWFQNRRMKWKK[11)]

[11)] Wherein 1-12 amino acid residues is deleted or variants thereof,

crrrrrrrr[12)]

[12)] Wherein 1-6 amino acid residues is deleted or variants thereof,

KWKKKWKKGGC[13)]

[13)] Wherein 1-6 amino acid residues is deleted or variants thereof,

RWRRRWRRGGC[14)]

[14)] Wherein 1-6 amino acid residues is deleted or variants thereof,

CFLPLIGRVLSGIL[15)]

15) Wherein 1-9 amino acid residues is deleted or variants thereof, ckkwkmrrnqfwvkvqr 16)

16) Wherein 1-12 amino acid residues is deleted or variants thereof, CGWTLNSAGYLLGKIN 17)

17) Wherein 1-11 amino acid residues is deleted or variants thereof, LKALAALAKKIL 18)

18) Wherein 1-7 amino acid residues is deleted or variants thereof, CALYLAIRRR 19)

19) Wherein 1-5 amino acid residues is deleted or variants thereof, YRRRFSVSVR 20)

20) Wherein 1-5 amino acid residues is deleted or variants thereof, RRLSYSRRRF 21)

21) Wherein 1-5 amino acid residues is deleted or variants thereof, KKFKVKFVVKK 22)

22) Wherein 1-7 amino acid residues is deleted or variants thereof, INLKALAALAKKIL 23)

23) Wherein 1-9 amino acid residues is deleted or variants thereof, AGYLLGKINLKALAALAKKIL 24)

24) Wherein 1-16 amino acid residues is deleted or variants thereof, VFQFLGKIIHHVGNFVHGFSHVF 25)

25) Wherein 1-18 amino acid residues is deleted or variants thereof, LFKRHLKWKIIV 26)

26) Wherein 1-7 amino acid residues is deleted or variants thereof.

6. A transporter peptide selected from:

KFLKLLKLFK

KLLKFFKFFK

KFAKAAKAFK

KFFKLLKFFK

KLFKLLKLFK

KFFKFFKLLK

KAFKAAKAFK

KVLKFFKFFK

KLLKFLKLFK

KFFKVLKFFK

KAAKFAKAFK

KFFKFFKVLK

KLLKLFKLFK

KLLKLLKFFK

KAAKAFKAFK

KLLKFFKLLK

KFFKLLKLLK

KVLKVLKFFK

KVLKFFKVLK

KFFKVLKVLK

KAAKAAKFFK

KAAKFFKAAK

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KLVVKFFK
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KAVVKFFK

KVAVKFFK
KVVAKFFK
KAAVKFFK
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KVAAKFFK
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KFFKLLLVK
KFFKVVLLK
KFFKVLVLK
KFFKVLLVK
KFFKLVVLK
KFFKLVLVK
KFFKLLVVK
KFFKVVVLK
KFFKVVLVK
KFFKLVVVK
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KFFKVAAVK
KFFKVAVAK
KFFKVVAAK
KFFKLLLK
KFFKVLLK

KFFKLVLK
KFFKLLVK
KFFKVVLK
KFFKVLVK
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KFFKAVVK
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KFFKAKVVV
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KFFKLKLL
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KFFKLKVL
KFFKLKLV
KFFKVKVL
KFFKVKLV
KFFKLKVV
KFFKVKVV
KFFKAKVV
KFFKVKAV
KFFKVKVA
KFFKAKAV
KFFKAKVA
KFFKVKAA

7. A transporter peptide selected from a peptide of formula $X_1X_2X_2X_2X_2X_1X_1X_2X_2X_1$, $X_1X_2X_2X_1X_1X_2X_2X_2X_2X_1$, or $X_1X_2X_2X_1X_2X_1X_1X_2X_2X_2$, wherein $X_1$ is K, R, E, D or H and $X_2$ is F, Y, I, L, V or A.

8. Use of a compound of any of the claims 1 to 7 for inactivation of the expression of specific genes by targeting the genes at the mRNA, sRNA or DNA level.

9. A method of treating a disease selected from bacterial and viral infections, cancer, metabolic diseases or immunological disorders comprising administering to a patient in need thereof an efficient amount of a compound of claim 1 to 7.

9. A compound of claim 1 to 7 for use in medicine.

10. A modified PNA molecule according to claim 1 to 4, wherein the PNA sequence is complementary to at least one nucleotide sequence in a bacteria.

11. A modified PNA molecule according to claim 10 wherein said nucleotide sequence is a ribosomal RNA, messenger RNA or DNA sequence.

12. A modified PNA molecule according to any of the claims 1 to 4, 10 or 11 , wherein the PNA sequence is in a parallel or anti-parallel orientation.

13. A modified PNA molecule according to any of the claims 10 to 12, wherein the functioning of the said nucleotide sequence is essential for the growth or survival of the bacteria and said functioning is blocked by the PNA sequence.

14. A modified PNA molecule according to any of the claims 1 to 4 or 10 to 13 for uses in the treatment of infectious diseases or in disinfection of non-living objects.

15. Use of a modified PNA molecule according to any of claims 1 to 4 or 10 to 13 in the manufacture of a medicament for the treatment of infectious diseases.

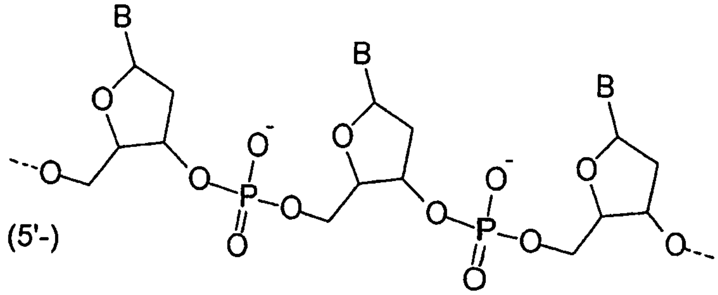
16. Use of a modified PNA molecule according to any of claims 1 to 4 or 10 to 13 in the manufacture of a composition for the treatment or prevention of bacterial infections.

17. A composition for use in the treatment or prevention of bacterial growth or survival, comprising a modified PNA molecule according to any of claims 1 to 4 or 10 to 13.

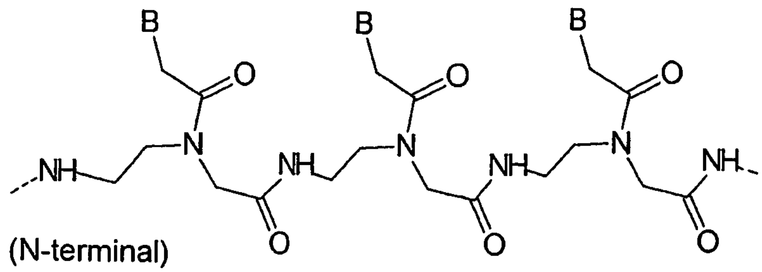
18. A composition according to claim 17 further comprising an antibiotic.

19. A composition according to claim 17 or 18 comprising two or more modified PNA molecules according to claims 1 to 4 or 10 to 13.

20. A method of treating an infectious disease, comprising administering to a patient in need thereof an efficient amount of a modified PNA molecule according to claims 1 to 4 or 10 to 13 or a composition according to any of claims 17 to 19.

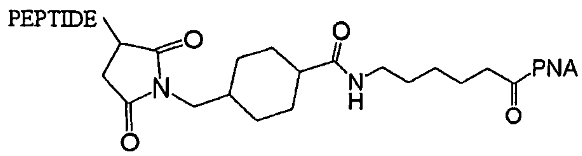



**DNA**

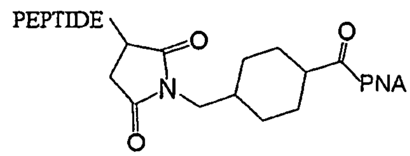



**PNA**

FIGURE 1



lc-smcc



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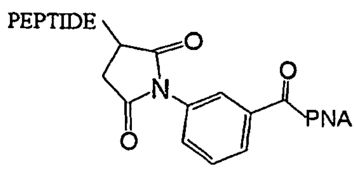
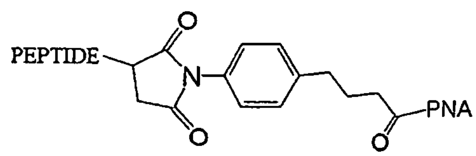
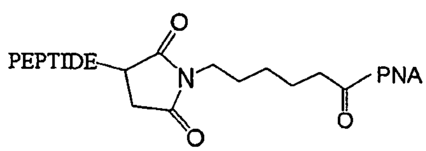


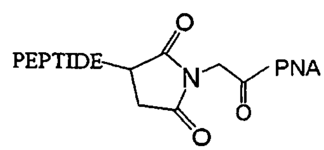
mbs



smpb



emcs



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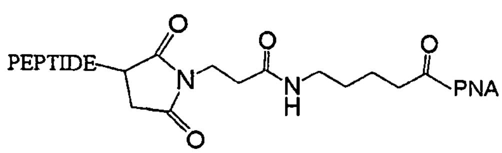

smph

FIGURE 2